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A Research Note

Vibrio parahaemolyticus in Long Island Oysters

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ABSTRACT

Twelve of 36 samples of Long Island oysters were found to contain *Vibrio parahaemolyticus* with a most probable number range of 3.6 to 23 organisms/g. Six of 10 isolates tested were weakly Kanagawa positive. None was pathogenic by the rabbit ileal loop test.

Vibrio parahaemolyticus, a facultative halophile commonly found in the marine environment, (*5-8,12,13*) has caused numerous outbreaks of gastroenteritis throughout the world following ingestion of raw or improperly cooked shellfish (*3,4*). Investigators have shown a seasonal variation in the numbers of *V. parahaemolyticus* detected in samples of water and shellfish from the Chesapeake Bay (*7*), Cape Cod (*5*) and Galveston Bay (*13*) areas. Higher concentrations occur in waters during the warmer months.

There are no reports of the incidence of *V. parahaemolyticus* in shellfish from the New York coastal area. Although a Kanagawa-positive strain was isolated from shellfish eaten by a Long Island woman who suffered acute gastroenteritis (*10*), the shellfish implicated originated from the Chesapeake Bay area. The purpose of the present study was to determine if *V. parahaemolyticus* was present and represented a potential public health problem in oysters harvested from growing areas off the Long Island coast.

MATERIALS AND METHODS

Inspectors of the Food and Drug Administration (FDA) collected oysters (ca 100/sample) from 17 different sampling sites representing 9 growing areas. Refrigerated samples were packed in shipping cartons or burlap bags and transported to the New York Regional FDA Laboratory in insulated containers. Samples were either analyzed immediately, or stored at 5 C and examined within 24 h of collection. The oysters were washed and shucked according to methods described in *Recommended Procedures for the Examination of Sea Water and Shellfish* (*1*).

All testing procedures were as outlined in chapter IX of the *Bacteriological Analytical Manual* (BAM) (2) except that the Analytical Profile Index (API) system (Analytab Products, Plainview, N.Y.) was employed to determine biochemical characteristics of the isolates. In brief, a 50-g portion of each sample was blended for 1 min with 450 ml of 3% sterile NaCl and three additional dilutions (10^{-2}-10^{-4}) were prepared. Three 1-ml portions of each dilution were inoculated into tubes containing 10 ml of single strength glucose salt Teepol (GST) broth (*2*). GST consists of 0.3% beef extract, 1% peptone, 3% NaCl, 0.5% glucose, 0.0002% methyl violet and 0.4% Teepol (Shell Chemical Co., Houston, Texas) with a final pH of 7.4. In addition three 10-ml portions of the 10^{-1} dilution were inoculated into 10 ml each of double strength GST broth.

After incubation overnight at 35 C, all tubes exhibiting growth were streaked onto plates of thiosulfate-citrate-bile salts-sucrose agar (Difco, Detroit, Michigan) and incubated for 18 h at 35 C. Blue-green colonies (sucrose-negative) with dark centers were picked (three per plate) as suspect *V. parahaemolyticus* and inoculated onto slants of triple sugar iron (TSI) agar (Difco) and heart infusion (HI) agar (Difco). Isolates producing an alkaline slant and acid butt with no gas or H_2S on TSI were inoculated from HI slants into API biochemical strips for presumptive identification. Motility, growth in salt trypticase broth (STB-1% trypticase, 0.3% yeast extract) (*2*) containing 0,6,8 and 10% NaCl, and growth at 42 C were also determined to confirm isolates as *V. parahaemolyticus*. Cultures so identified were forwarded to R. M. Twedt, Division of Microbiology, FDA, Cincinnati, OH for determination of the Kanagawa reaction. All Kanagawa-positive isolates were tested for pathogenicity in rabbits by the ileal loop procedure (*11*).

RESULTS AND DISCUSSION

From October, 1979 to June, 1980, a total of 36 oyster samples were analyzed and 12 (33%) were found to contain *V. parahaemolyticus* with a most probable number range of 3.6 to 23 organisms/g (Table 1). A total of 36 isolates were tested and all exhibited reactions typical for *V. parahaemolyticus* as listed in BAM with the exception of one isolate which fermented sucrose, and 7 isolates which grew in STB with 0 and 10% NaCl. Liston and Baross (*8*) reported that only about 17% of *V. parahaemolyticus* strains ferments sucrose. Positive samples were obtained from only 7 of 17 sampling sites. Recovery was limited to the months of October, November, May and June when water temperatures were high enough to permit the survival of *V. parahaemolyticus*. Samples were not collected in July and August. Our results agree with those of Kaneko and Colwell (*7*) who showed that *V. parahaemolyticus* survives in sediment during winter months and is recovered from water after the water temperature rises sufficiently.

TABLE 1. V. parahaemolyticus *in Long Island oysters.*

Sampling site	Sampling date	MPN/g[a]
Oyster Bay #1	10/79	3.6
Northport	10/79	3.6
	11/79	<3
Millneck Creek #1	10/79	<3
Millneck Creek #2	11/79	9.3
Oyster Bay #2	11/79	9.3
	12/79	<3
Sag Harbor #1	11/79	<3
	12/79	<3
Oyster Bay #3	1/80 (2 samples)	<3,<3
	3/80	<3
	4/80	<3
Cedar Beach	1/80	<3
	2/80 (2 samples)	<3,<3
Peconic Bay	1/80	<3
Oyster Bay #4	2/80	<3
Oyster Bay #5	2/80	<3
East Marion	3/80	<3
Oyster Bay #6	4/80 (2 samples)	<3,<3
	5/80 (3 samples)	<3,9.3,3.6
Sag Harbor #2	4/80	<3
Long Beach	5/80 (4 samples)	<3,<3,23,23
	6/80 (2 samples)	23,23
Oyster Bay #7	5/80	<3
	6/80 (2 samples)	9.1,9.1
Fiddler's Green	6/80	<3

[a]MPN, most probable number by the three-tube procedure.

Serotyping was performed on only one isolate (identified as K4). Six isolates exhibited some hemolytic activity and were thus considered to be weakly Kanagawa-positive and potentially pathogenic. However, none of the 10 isolates tested, including the 6 Kanagawa-positive strains, proved to be pathogenic by the rabbit ileal loop test. Although it is believed that positive Kanagawa reactions are directly related to pathogenicity (*3*) this must not be the case with the weakly positive strains detected here. Our results correlate with those of other investigators (*3,10*) in that environmental isolates have not proven to be pathogenic, perhaps, as suggested by Hackney et al. (*6*), because presently employed isolation procedures favor non-pathogenic strains.

The levels of *V. parahaemolyticus* detected in the present limited survey are below those found by others. A cooperative survey of market finfish, shellfish and shrimp was recently conducted by 10 Japanese public health laboratories in order to identify the extent of *V. parahaemolyticus* contamination and recommend a hygenic standard for food safety (*9*). *V. parahaemolyticus* was recovered from 74 of 143 (51.7%) shellfish samples examined and mean MPN values ranging from $<10^2$ to $>10^5$/100 g were found. In some cases, just after harvest, shellfish specimens contained as many as 10^6 *V. parahaemolyticus*/100 g. Whereas an acceptance limit of 10^4 *V. parahaemolyticus*/100 g of finfish was recommended based on market contamination rates, they concluded that this level was not yet attainable in shellfish.

In light of these Japanese findings, the low level of *V. parahaemolyticus* recovered from Long Island oysters does not appear to pose a public health hazard. Refrigeration of our samples during transportation and short-term storage (<24 h) may have contributed to the lower counts observed in the survey. Nevertheless, since these handling methods duplicate commercial procedures, they could be expected to accurately assess the hazard to the consumer of Long Island oysters.

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